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# Spectrum of Zygomycete Species Identified in Clinically Significant Specimens in the United States▽†

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**Several members of the order *Mucorales* (subphylum *Mucoromycotina*) are important agents of severe human infections. The identification of these fungi by using standard mycologic methods is often difficult and time consuming. Frequently, the etiological agent in clinical cases is reported either as a *Mucor* sp., which is not the most frequent genus of zygomycetes, or only as a member of the *Mucorales*. For this reason, the actual spectrum of species of zygomycetes and their incidences in the clinical setting is not well known. The goals of this study were to compare the results of the molecular identification of an important set of clinical isolates, received in a mycological reference center from different regions of the United States, with those obtained by using the traditional morphological methods and to determine the spectrum of species involved. We tested 190 isolates morphologically identified as zygomycetes by using sequencing of the internal transcribed spacer (ITS) region of the ribosomal DNA. Molecular identification revealed that *Rhizopus oryzae* represented approximately half (44.7%) of these isolates. The remainder was identified as *Rhizopus microsporus* (22.1%), *Mucor circinelloides* (9.5%), *Mycocladus corymbifer* (formerly *Absidia corymbifera*) (5.3%), *Rhizomucor pusillus* (3.7%), *Cunninghamella bertholletiae* (3.2%), *Mucor indicus* (2.6%), *Cunninghamella echinulata* (1%), and *Apophysomyces elegans* (0.5%). The most common anatomic sites for clinically significant zygomycetes, as determined by isolates sent to the Fungus Testing Laboratory for identification and/or susceptibility testing and included in this study, were the sinuses, lungs, and various cutaneous locations, at 25.8%, 26.8%, and 28%, respectively. These sites represented approximately 80% of the isolates evaluated. A high level of correlation (92.6%) between morphological and molecular identifications was found.**

Members of the subphylum *Mucoromycotina* (formerly *Zygomycota*) (10) are characterized by the production of a coenocitic mycelium and the formation of asexual spores (sporangiospores) in a variety of fungal structures. A few are homothallic, forming zygospores in culture. They are distributed worldwide and are ubiquitous in soil and organic substrates. Roden et al. (23) reported a 70% increase in the number of cases of zygomycosis between 1940 and 2000. These infections were more frequently seen in neutropenic patients, transplant recipients, patients with hematological disease or diabetes mellitus, patients receiving deferoxamine therapy (9, 18, 21, 32, 38), and intravenous drug users (17). The most common clinical infections in order were rhino-orbito-cerebral, cutaneous, pulmonary, disseminated, and gastrointestinal manifestations (23). The most clinically important zygomycetes are in the order *Mucorales*, comprising approximately 60 genera, some of which are important etiologic agents of human disease, especially in immunocompromised patients (6). *Rhizopus* is the most common genus causing human infection, although other genera such as *Mucor*, *Rhizomucor*, *Cunninghamella*, *Apophysomyces*, and *Mycocladus* (formerly *Absidia*) have also been reported, although less frequently (6, 7, 21).

As has been demonstrated, pathogenic species of the zygomycetes show important differences in their responses to antifungal drugs (1, 31), and their correct identification in human infection is of prime importance (4). However, the etiologic agents of zygomycoses in numerous clinical cases are not identified to the species level or are more commonly being improperly named *Mucor* spp. Routine laboratory tests commonly identify isolates only as a zygomycete or to the genus level at best. In the most comprehensive review of zygomycoses published to date, a high percentage of the 929 cases reviewed lacked identification to the species level, and for most, the identification is doubtful (23). In recent years, it has been demonstrated that the analysis of DNA sequences, especially that of ribosomal DNA (rDNA), is very useful for the identification of zygomycetes (29, 36, 37).

We have retrospectively analyzed a large number of human clinical isolates of zygomycetes preserved at the Fungus Testing Laboratory in the Department of Pathology at the University of Texas Health Science Center at San Antonio. Given the difficulty of morphological identification, final identifications were reached after sequencing the internal transcribed spacer (ITS) region of the rDNA.

## MATERIALS AND METHODS

**Fungal isolates.** A total of 190 clinical isolates submitted to the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio from the period of January 2001 to February 2007 were analyzed (see the supplemental material). In addition, the type or reference strains of *Apophysomyces elegans*, *Cunninghamella bertholletiae*, *Cunninghamella echinulata*, *Mucor circinelloides*, *Mucor hiemalis*, *Mucor indicus*, *Mucor racemosus*, *Mucor ramosissimus*, *Mycocladus corymbifer*, *Rhizomucor pusillus*, *Rhizomucor variabilis*, *Rhizo-*

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TABLE 1. Comparison of morphological and molecular identification of isolates examined

| Morphological identification of species | No. of isolates identified by molecular methods | | | | | | | | | | | | | | | % Correlation (morphological/ molecular) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *R. oryzae* | *R. microsporus* | *M. corymbifer* | *M. circinelloides* | *R. pusillus* | *C. bertholletiae* | *M. indicus* | *Apophysomyces* spp. | *Mucor* sp. strain 1[a] | *Mucor* spp. | *Cunninghamella* spp. | *C. echinulata* | *Mucor* sp. strain 2[b] | *A. elegans* | Total | |
| *Rhizopus oryzae* | 85 | | | | | | | | | | | | | | 85 | 100 |
| *Rhizopus microsporus* | | 42 | | | | | | | | | | | | | 42 | 100 |
| *Mucor circinelloides* | | | | 18 | | | | | | | | | | | 18 | 100 |
| *Mycocladus corymbifer* | | | 10 | | | | | | | | | | | | 10 | 100 |
| *Cunninghamella bertholletiae* | | | | | | 6 | | | | | 3 | | | | 9 | 66 |
| *Rhizomucor pusillus* | | | | | 7 | | | | | | | | | | 7 | 100 |
| *Mucor indicus* | | | | | | | 5 | | | | | | 1 | | 6 | 83.3 |
| *Apophysomyces elegans* | | | | | | | | 4 | | | | | | 1 | 5 | 20 |
| *Mucor racemosus* | | | | | | | | | | 3 | | | | | 3 | 0 |
| *Mucor ramosissimus* | | | | | | | | | 3 | | | | | | 3 | 0 |
| *Cunninghamella echinulata* | | | | | | | | | | | | 2 | | | 2 | 100 |
| Total | 85 | 42 | 10 | 18 | 7 | 6 | 5 | 4 | 3 | 3 | 3 | 2 | 1 | 1 | 190 | 92.6 |

[a] The results of the BLAST search showed 94 to 95% MLI with the *Mucor circinelloides* type strain CBS 195.68 and 97 to 99% MLI with a *Mucor racemosus* non-type strain (ATCC 1216B; AJ271061.1). In the phylogenetic tree (Fig. 2), the sequences of this species and of the *Mucor racemosus* type strain were rather distant.
[b] The results of the BLAST search showed 95% MLI with the *Mucor indicus* type strain CBS 226.29.

*mucor variabilis* var. *regularior*, *Rhizopus microsporus*, and *Rhizopus oryzae* were also included in the analysis. All isolates, including the reference strains, were subcultured onto plates containing potato dextrose agar (Pronadisa, Madrid, Spain) at room temperature (24°C ± 2°C) for 2 to 5 days to ensure purity. Isolates were subsequently grown on potato dextrose agar slants for 7 days and later covered with mineral oil for long-term room temperature storage.

**Morphological identification.** The isolates were identified using schemes based on morphological and physiological characters (6, 25–28, 40). Microscopic observation was made from slide preparations mounted in water and lactophenol, using a Leitz Dialux 20EB microscope.

**DNA extraction, amplification, and sequencing.** DNA was extracted and purified directly from fungal colonies following the FastDNA kit protocol (Bio101, Vista, CA), with a minor modification consisting of a homogenization step repeated three times with a FastPrep FP120 instrument (Thermo Savant, Holbrook, NY). The DNA was quantified by GeneQuant *pro* (Amersham Pharmacia Biotech, Cambridge, England). The ITS region of the nuclear rDNA was amplified with the primer pair ITS5 and ITS4, following the protocol described by Gilgado et al. (8).

The PCR mix (25 μl) included 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$ (10× PerkinElmer buffer II plus $MgCl_2$ solution; Roche Molecular Systems, Branchburg, NJ), 100 μM of each deoxynucleoside triphosphate (Promega, Madison, WI), 1 μM of each primer, and 1.5 U of AmpliTaq DNA polymerase (Roche). The amplification program included an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing for 1 min at 52°C, and extension for 1 min at 72°C. After PCR analysis, the products were purified with an illustra GFX PCR DNA and gel band purification kit (General Electric Healthcare, Buckinghamshire, United Kingdom) and stored at −20°C until they were used in sequencing. PCR products were sequenced by using the same primer used for amplification and following the *Taq* DyeDeoxy Terminator cycle sequencing kit protocol (Applied Biosystems, Gouda, The Netherlands). Reactions were run on a 310 DNA sequencer (Applied Biosystems). Consensus sequences were obtained using the AutoAssembler program (PerkinElmer-Applied Biosystems) and SeqMan software (Lasergene, Madison, WI) Multiple-sequence alignments were performed with the ClustalX (version 1.8) computer program (35), followed by manual adjustments with a text editor. These products were analyzed on an ABI Prism 310 automated DNA analyzer (Applied Biosystems).

**Phylogenetic analyses.** The phylogenetic analyses were performed with the help of the software program MEGA 4.0 (33). The maximum composite likelihood algorithm was used for the determination of evolutionary distances between sequences. Trees were built using the neighbor-joining (NJ) method. Gaps were treated as pairwise deletions. Support for internal branches was assessed by a search of 1,000 bootstrapped sets of data. The final identification of the isolates was performed using Basic Local Alignment Search Tool (BLAST) searches (2). Only the nucleotide sequences of type or reference strains, deposited in the GenBank/EMBL database, were considered for identification purposes. When the BLAST algorithm aligned and compared the sequences of our isolates with those considered confident, a maximal level of identity (MLI) equal to or higher than 98% was considered for specific identification. MLI values lower than 98% provided identification only to the genus level.

**Nucleotide sequence accession numbers.** Sequences from one isolate identified as *Mycocladus corymbifer* (UTHSC 06-1655) and another identified as *Rhizopus microsporus* var. *oligosporus* (UTHSC 03-3512) have been deposited in GenBank under accession numbers FN293105 and FN293106, respectively.

## RESULTS

The lengths of the amplicons of the ITS1-5.8S-ITS2 region in *Rhizopus oryzae* isolates varied from 530 to 550 bp; in *R. microsporus* isolates, from 601 to 616 bp; in *Mucor circinelloides*, from 524 to 572 bp; in *Mycocladus corymbifer*, from 681 to 700 bp; in *Cunninghamella bertholletiae* and a *Cunninghamella* sp., from 602 to 631 bp; in *Mucor indicus*, from 520 to 537 bp; in *Rhizomucor pusillus*, from 526 to 529 bp; in *Apophysomyces elegans* and an *Apophysomyces* sp., 672 to 818 bp; in the other *Mucor* sp., from 524 to 572 bp; and in *Cunninghamella echinulata*, from 770 to 810 bp.

Table 1 shows the results of the molecular identification. A total of nine known species could be identified. In addition, two undescribed species of *Mucor* (called *Mucor* sp. strain 1 and *Mucor* sp. strain 2), which were morphologically identified as *Mucor ramosissimus* and *Mucor indicus*, respectively, were also detected. The molecular study also revealed that not all the *Apophysomyces* isolates belong to a single species, but unfortunately the ITS sequence of the type strain of *A. elegans* is not in GenBank and was not available for study. Lastly, molecular characterization also supports the possibility of other species of *Cunninghamella* in addition to *C. echinulata*

and *C. bertholletiae*. In order of frequency, the most prevalent agent of zygomycosis was *Rhizopus oryzae*, comprising nearly half of the isolates tested (44.7%), and this was followed by *R. microsporus* (22.1%), *Mucor circinelloides* (9.5%), *Mycocladus corymbifer* (5.3%), *Rhizomucor pusillus* (3.7%), *Cunninghamella bertholletiae* (3.2%), *Mucor indicus* (2.6%), *Cunninghamella echinulata* (1%), and *Apophysomyces elegans* (0.5%). Since only a total of 7.4% of the isolates could not be identified to the species level, the correlation between morphological and genetic methods at the species level was 92.6% and at the genus level it was 100%. The only discrepancies corresponded with those isolates that represented undescribed species. A listing of the anatomic sites for the isolates based upon the information available and cross-referenced by species is provided in Table 2. The majority of the isolates, approximately 80%, were represented by isolates from the sinuses (25.8%), lungs (26.8%), and various cutaneous presentations (28%). The remaining 20% of the isolates consisted of a subset collected from deep sites, such as the brain, bones, liver, bladder, blood, bowel, and heart as well as from a few miscellaneous sites and one isolate from a marine mammal.

Figure 1 shows the NJ tree of the 5.8S rRNA genes of a representative number of the isolates treated in this study, including the type and reference strains of the species mentioned above. Due to the high level of variability in the sequences of the ITS regions, it was not possible to align them all with confidence. Therefore, in this analysis we used only sequences of the 5.8S rRNA gene. Six main clades, each supported by a high bootstrap value and representing a different genus (*Mucor*, *Rhizopus*, *Mycocladus*, *Apophysomyces*, *Rhizomucor*, and *Cunninghamella*), were observed in the phylogenetic tree (Fig. 1). The different species of each genus were also well separated, with the exception of those belonging to the genus *Mucor*. To better determine the phylogenetic relationship among the species of the genus *Mucor*, a new analysis using the sequences of the ITS regions of the isolates of this genus was performed. The sequences of the type strains of *Rhizomucor variabilis* var. *variabilis* and *Rhizomucor variabilis* var. *regularior* were also included to build an NJ tree (Fig. 2). In the ITS tree, the *Mucor* species were well separated in different clades, with each receiving a high level of statistical support. All the isolates of *M. circinelloides* and the type strain of *Rhizomucor variabilis* var. *regularior* were nested in a single clade. A total of 14 isolates could not be assigned to any known species.

TABLE 2. Source and identification of isolates examined

| Species | No. (%) of isolates obtained from indicated source(s) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Most common | | | | Systemic | | | | | | | | | | Miscellaneous | | | |
| | Sinuses and related sites | Lungs and related sites | Various cutaneous sites | Total | Brain | Bone | Liver | Urine/bladder | Blood | Peritoneal fluid | Bowel | Heart | Unknown tissue | Nail | Genitals | Unknown tissue | Dolphin | Grand total (%) |
| *Rhizopus oryzae* | 32 | 14 | 26 | 72 | | 1 | | 2 | | | 1 | | 6 | 1 | 1 | 1 | | 85 (44.7) |
| *Rhizopus microsporus* | 5 | 22 | 10 | 34 | | 1 | | 1 | | | | | 1 | | | 2 | | 42 (22.1) |
| *Mucor circinelloides* | 4 | 2 | 7 | 13 | | 1 | | | 1 | | | | 1 | | | 2 | | 18 (9.5) |
| *Mycocladus corymbifer* | 3 | 3 | | 7 | 1 | | | | 1 | | | 1 | | | | | | 10 (5.3) |
| *Rhizomucor pusillus* | 2 | 3 | 1 | 6 | | | | | 3 | 1 | | | | | | 1 | | 7 (3.7) |
| Other *Mucor* spp. | 1 | 1 | 2 | 4 | | | | | | | | | | | | | | 7 (3.7) |
| *Cunninghamella bertholletiae* | 1 | 3 | 1 | 5 | 1 | | | | | 1 | | | | | | | | 6 (3.2) |
| *Mucor indicus* | | | 1 | 1 | | | | | | 1 | | | 1 | | | | | 5 (2.6) |
| Other *Apophysomyces* spp. | 1 | | 3 | 4 | | | 2 | | | | | | | | | | | 4 (2.1) |
| Other *Cunninghamella* spp. | 1 | 2 | | 3 | | | | | | | | | | | | | | 3 (1.6) |
| *Cunninghamella echinulata* | 1 | 1 | 1 | 3 | | | | | | | | | | | | | | 2 (1) |
| *Apophysomyces elegans* | | | | | | | | | | | | | | | | | 1 | 1 (0.5) |
| Total | 49 (25.8) | 51 (26.8) | 53 (28) | 153 (80.6) | 2 (1.1) | 3 (1.6) | 2 (1.1) | 3 (1.6) | 5 (2.6) | 2 (1.1) | 1 (0.5) | 1 (0.5) | 9 (4.7) | 1 (0.5) | 1 (0.5) | 6 (3.1) | 1 (0.5) | 190 (100) |

## DISCUSSION

This study contains the largest number of clinical isolates of zygomycetes identified to the species level by molecular characterization. Unfortunately, similar studies performed in other countries for comparison purposes do not exist. In the review of Roden et al. (23), the zygomycetes causing approximately half of the reported cases were identified by culture. However, that study was only a compilation of unrelated cases, with identifications performed in different institutions. In numerous cases, the mycological methods used were not described. Therefore, most of those identifications are considered doubtful or of insufficient accuracy. Three recent retrospective studies performed in the United States (13, 14, 30) included a

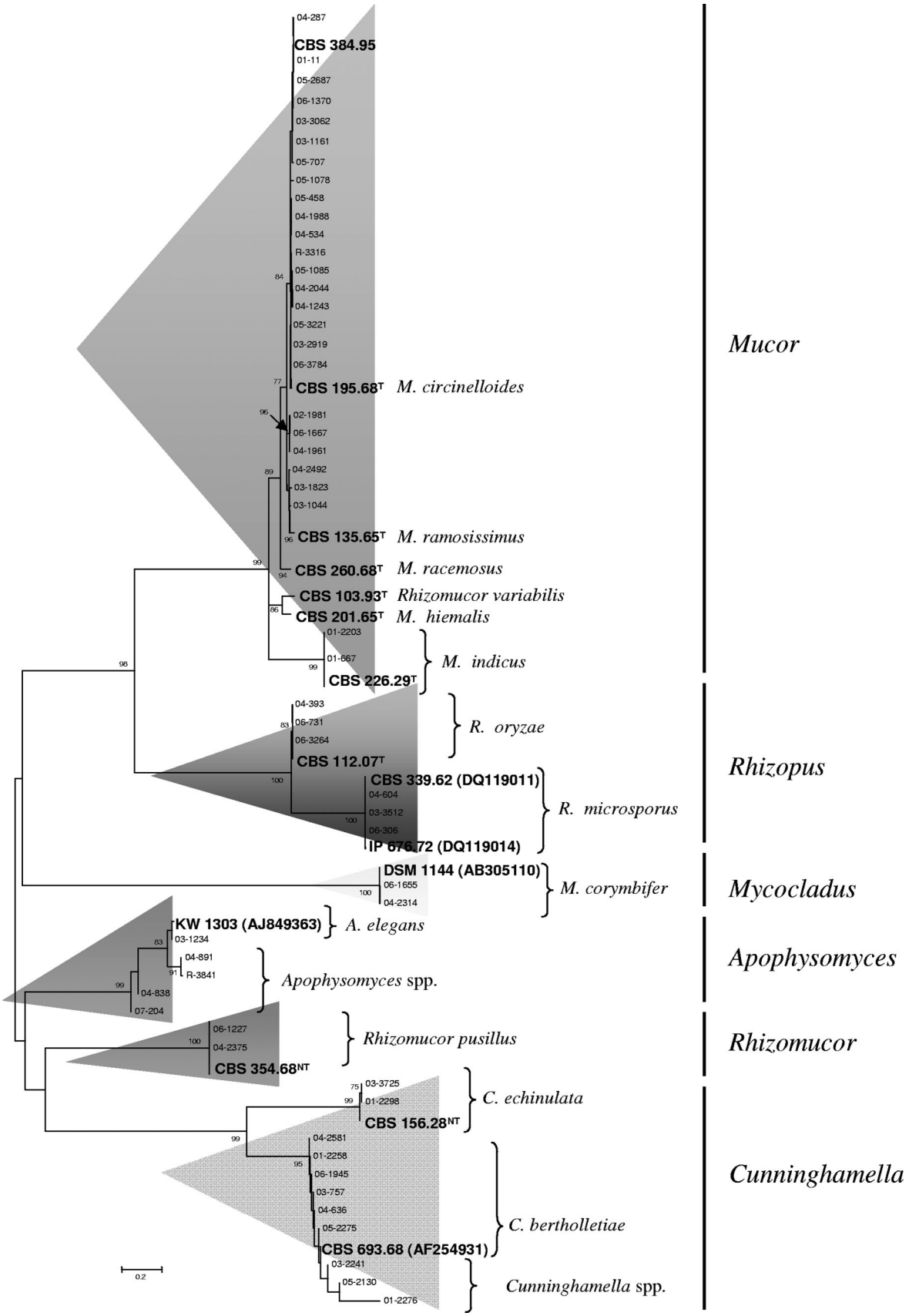
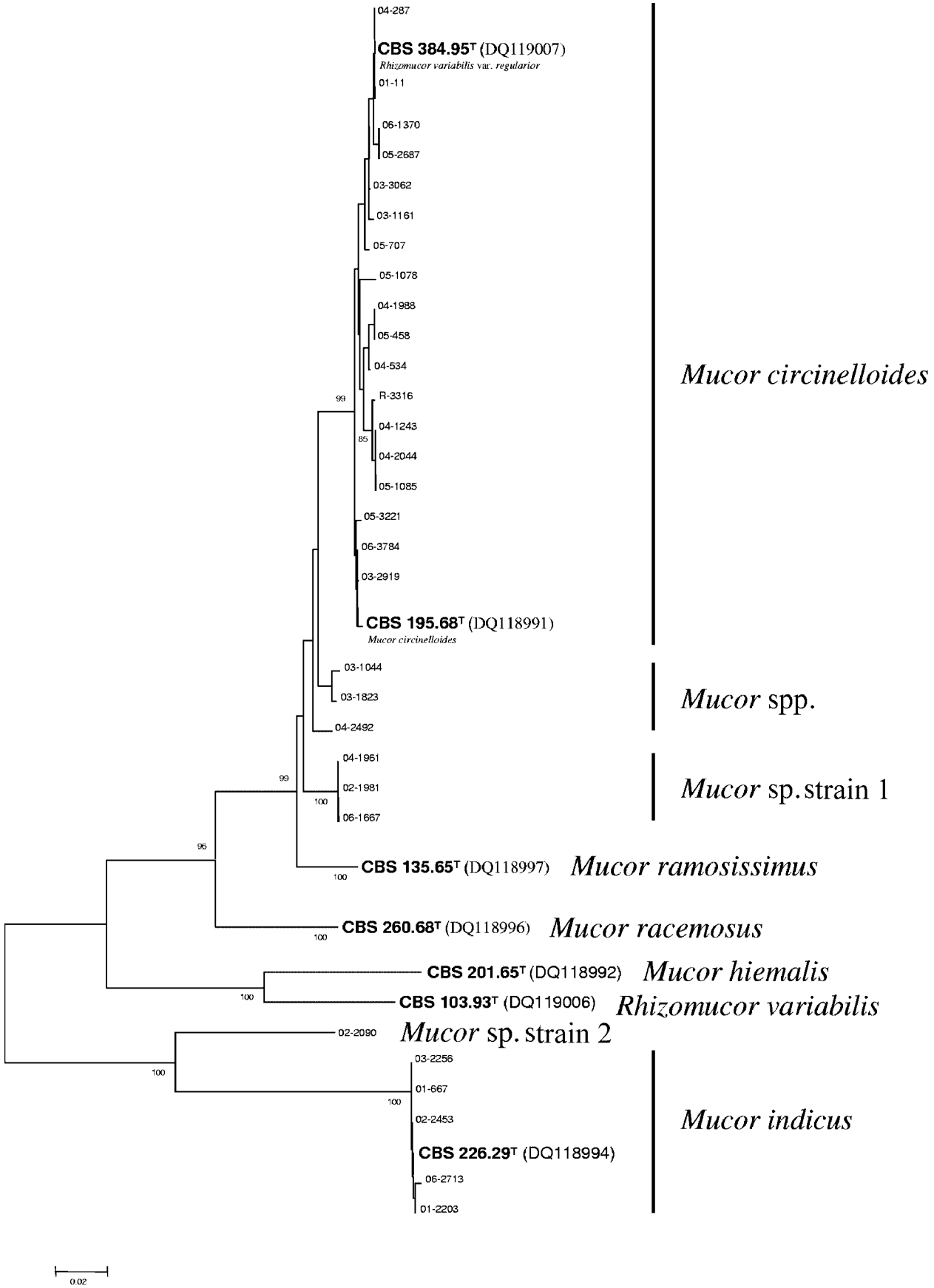



FIG. 1. NJ tree based on maximum composite likelihood-corrected nucleotide distances among 5.8S rRNA gene sequences of representative isolates of the species listed in Table 1. In the tree, branch lengths are proportional to distance. Bootstrap iteration frequencies (1,000 iterations) above 70% are indicated on the nodes. Type or reference strains are indicated in boldface. $^{T}$, type strain; $^{NT}$, neotype strain.


04-287
CBS 384.95T (DQ119007)
Rhizomucor variabilis var. regularior
01-11
06-1370
05-2687
03-3062
03-1161
05-707
05-1078
04-1988
05-458
04-534
R-3316
04-1243
04-2044
05-1085
05-3221
06-3784
03-2919
CBS 195.68T (DQ118991)
Mucor circinelloides
Mucor circinelloides
03-1044
03-1823
04-2492
Mucor spp.
04-1961
02-1981
06-1667
Mucor sp. strain 1
CBS 135.65T (DQ118997) Mucor ramosissimus
CBS 260.68T (DQ118996) Mucor racemosus
CBS 201.65T (DQ118992) Mucor hiemalis
CBS 103.93T (DQ119006) Rhizomucor variabilis
02-2090
Mucor sp. strain 2
03-2256
01-667
02-2453
CBS 226.29T (DQ118994)
06-2713
01-2203
Mucor indicus
99
85
99
100
96
100
100
100
100
100
0.02

significant number of cases. However, one of these (30) distinguished only between *Mucor* spp. and *Rhizopus* spp., and in the others (13, 14) only the genus names were mentioned (*Cunninghamella*, *Mucor*, *Rhizopus*, and *Syncephalastrum*). The election of a cutoff score of ≥98% for the molecular identification of the isolates was based in the sequence variability observed within the species represented by well-supported clades (bootstrap values, ≥80%) in the phylogenetic tree (Fig. 1).

In this study, isolates from the sinuses and related areas (sino-orbital, sino-nasal, and hard palate) represented 25.8% of the isolates evaluated. Roden et al. (23) reported that sinus involvement, consisting of rhinocerebral, sinus, and sino-orbital infections, constituted the majority (66%) of infections in diabetic patients.

The lungs and related sites were similarly represented at 26.8%. Among all forms of zygomycosis, cutaneous infection is a less frequent presentation (3, 21), and it is associated with penetrating trauma, burns, motor vehicle accidents, and falls (23). In the present study, however, cutaneous sites accounted for 28% of the isolates, and the most prevalent agent was *Rhizopus oryzae*.

The molecular identification of clinical zygomycetes using the ITS region has been successfully used in recent years (11, 12, 19, 29, 36, 37, 39). However, a BLAST search can constitute an important limitation of such procedures when comparisons are made with inaccurate sequence data (5). In our case for the sequences of *Mucor circinelloides*, the BLAST search gave a similarity of 99 to 100% with the type strain of *Rhizomucor variabilis* var. *regularior*. When we compared the sequences of the type strains of both species, we noticed that they were identical. Considering that in our phylogenetic tree the type species of *Rhizomucor*, *Rhizomucor pusillus*, was placed very far from the *Mucor* clade (Fig. 2), it seems logical to consider *R. variabilis* var. *regularior* a synonym of *Mucor circinelloides*. Schwarz et al. (29) also previously reported a high level of similarity between the sequences of these two species. *Rhizomucor variabilis* var. *variabilis* was also included in our study in the *Mucor* clade, in this case close to *M. hiemalis*. Voigt et al. (37), analyzing the 28S and 18S rDNA loci, also reported that *R. variabilis* var. *variabilis* was phylogenetically closely related to *Mucor hiemalis* and *Mucor mucedo*. The most important morphological feature reported in the literature to differentiate *Rhizomucor* spp. from *Mucor* spp. is the presence of rudimentary rhizoids in the former. However, this does not seem to be a very consistent taxonomic feature, since this study demonstrated that the two varieties of *R. variabilis*, which have such rhizoids, belong to the genus *Mucor*. In addition, *R. variabilis* shows several morphological features typical of *Mucor* spp., such as the size and type of sporangiospores, the presence of chlamydospores, the maximum temperature for growth, and other cultural characteristics.

Infections by *Rhizomucor* spp. are rare in humans and are caused mostly by *R. pusillus* (11, 21). The sequences of the *R. pusillus* isolates analyzed here were very similar, and they can be easily distinguished from those of other genera. These results agree with previous studies reported by different authors (11, 19, 29).

In the present study, the ITS sequences of six isolates included in the *Mucor* clade (Fig. 2) presented a very low level of similarity with the sequences of the species of *Mucor* deposited in GenBank. Three of these isolates showed identical sequences (*Mucor* sp. strain 1) and were distributed into one well-supported subclade. Initially they were morphologically identified as *Mucor ramosissimus*. However, the BLAST search showed a low percentage of similarity (94 to 95%) with the type strain of *M. ramosissimus* (CBS 135.65). The other three isolates, which had different sequences between them, were initially morphologically identified as *Mucor racemosus*. In this case, the BLAST search for these isolates also showed a low similarity (90 to 91%) with the type strain of *M. racemosus* (CBS 260.68). Another isolate of *Mucor* that we could not identify to the species level was UTHSC 02-2090 (*Mucor* sp. strain 2). This isolate was phylogenetically and morphologically related to *M. indicus* isolates. It is of interest that two of the *M. indicus* isolates were recovered from the liver, corroborating previous reports of this organism's ability to disseminate, particularly in immunocompromised and/or neutropenic individuals (34). One report of a bone marrow transplant recipient suggests that the organism may have been acquired following ingestion of naturopathic medicine containing the organism (20).

In general, we found a high genetic variability in the 5.8S rRNA gene sequences of species from *Cunninghamella* and *Apophysomyces*. In the former genus, our analysis was able to clearly differentiate *C. echinulata* and *C. bertholletiae*. However, we were not able to identify to the species level the other three isolates with high percentages of similarity which were morphologically identified as *C. bertholletiae*. The most common and practically the sole species of the genus *Cunninghamella* that is traditionally considered the etiologic agent of human infections is *C. bertholletiae* (21, 24). However, Lemmer et al. (15) reported in Germany a case of human infection by *C. echinulata* which was identified by sequencing the ITS region. In that study, the isolates of *C. echinulata* from environmental and clinical origins showed identical digestion patterns in the ITS restriction fragment length polymorphism by using TaqI and HinfI. Our study confirms the identification of *C. echinulata* as a clinically significant isolate. In the *Apophysomyces* clade, although all the isolates included were morphologically similar to *A. elegans*, the only species of the genus involved in human infections so far, a high level of molecular intraspecific variability was observed. Five of these isolates showed a low level of similarity (87 to 91%) with the sequence of a reference strain of *A. elegans* (AJ849363). Most of the *Apophysomyces elegans* infections reported have occurred in immunocompetent patients (16). In this study, the one isolate with 99% similarity to the reference strain was recovered from a dolphin. This organism, along with *Saksenaea vasiformis*, is a

FIG. 2. NJ tree based on maximum composite likelihood-corrected nucleotide distances among the ribosomal ITS regions and 5.8S rRNA gene sequences of the isolates included in the *Mucor* clade shown in Fig. 1. In the tree, branch lengths are proportional to distance. Bootstrap iteration frequencies (1,000 iterations) above 70% are indicated on the nodes. Type strains are indicated in boldface. $^{T}$, type strain.

known aggressive and commonly systemic pathogen in killer whales (*Orcinus orca*), Pacific white-sided dolphins (*Lagenorhynchus obliquidens*), and bottlenose dolphins (*Tursiops truncates*) (22). The other four morphologically similar human isolates occurred in one case of sino-orbital involvement and three cutaneous presentations (Table 2).

In conclusion, although the identification of zygomycetes remains a difficult and time-consuming task, this study has demonstrated that morphological features alone, when assessed by individuals with expertise in fungal identification, can provide a high level of accuracy and that ITS sequencing can be a useful tool in the identification of the most common clinically significant species of zygomycetes and the delineation of undescribed species. The most common species in this set of clinical isolates were *Rhizopus oryzae* and *Rhizopus microsporus*.

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